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Prevalence and Antibiotic Resistance of *Salmonella* spp. in Pigeons Farmed at Bac Ninh Province, Vietnam

Chu Thi Thanh Huong[1], Truong Lan Oanh[2], Vu Thi Thu Tra[2], and Truong Ha Thai[1]*

[1]*Department of Veterinary Microbiology and Infectious Diseases, Faculty of Veterinary Medicine, Vietnam National University of Agriculture, Gia Lam, Hanoi, Vietnam*
[2]*Department of Veterinary Public Health, Faculty of Veterinary Medicine, Vietnam National University of Agriculture, Gia Lam, Hanoi, Vietnam*

*Corresponding author's E-mail: ththai@vnua.edu.vn

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ABSTRACT

Pigeons are known to be the potential reservoir for several pathogenic microorganisms, including *Salmonella*, *E. coli*, *Chlamydia* spp., and *Cryptococcus* spp. This study aimed to determine the prevalence and antibiotic resistance of *Salmonella* in fecal samples from pigeons raised in Northern Vietnam. From January to December 2024, 128 fresh fecal samples were collected from 32 different pigeon farms in Bac Ninh province, Vietnam, for *Salmonella* isolation. Fecal samples were cultured on peptone buffer and selective media such as rappaport-vassilidis soybean broth and xylose lysine deoxycholate agar; suspected *Salmonella* colonies were confirmed by biochemical tests and *InvA* gene identification. Results showed that 40 out of 128 samples (31.3%) were positive for *Salmonella*. The isolated *Salmonella* strains were tested for antibiotic susceptibility by agar diffusion method for ampicillin, meropenem, cefotaxime, ceftazidime, doxycycline, tetracycline, gentamicin, streptomycin, nalidixic acid, ciprofloxacin, norfloxacin, sulfonamide, and trimethoprim/sulfamethoxazole. The isolates exhibited the highest resistance rate to tetracycline (67.5%), followed by ampicillin and sulfonamide (62.5%). Cefotaxime, streptomycin, and trimethoprim/sulfamethoxazole were resistant at rates of 52.0%, 47.5%, and 45.0%, respectively. Resistance to ciprofloxacin, gentamicin, doxycycline, nalidixic acid, and chloramphenicol ranged from 20.0% to 35.0%. No *Salmonella* strains were observed to be resistant to ceftazidime, meropenem, and norfloxacin. The current results indicated that 92.5% (37/40) isolates were resistant to at least one antibiotic, with 26 different antibiotic resistance patterns. Twenty-eight (70.0%) strains were identified as multidrug-resistant (MDR), with resistance to 3-4 antibiotic classes, and 5-6 antibiotic classes and 7 antibiotic classes found in 11 (27.5%), 12 (30.0%), and 5 (12.5%) of the isolates, respectively. Overall, the findings of the current study indicated a high prevalence of antibiotic-resistant *Salmonella* in pigeon farms, with many strains identified as MDR.

Keywords: Antibiotic resistance, *InvA*, Pigeon, *Salmonella*

INTRODUCTION

Salmonella enterica is a species of Gram-negative bacteria, a member of the *Enterobacteriaceae* family, and over 2,500 different serotypes have been identified (Grimont and Weill 2007). Notably, animals infected with *Salmonella*, including rats, wild animals, poultry, and cattle, are important sources of *Salmonella* infection in humans (Zhang et al., 2024).

Globally, pigeons (*Columba livia*) are highly valued for the high nutritional content of their meat and eggs. Therefore, the domestic pigeon farming industry has developed into an important part of the poultry industry in many countries around the world (Karim et al., 2020; Wang et al., 2025). In Vietnam, pigeons are increasingly raised for meat due to the low cost of housing, food, and veterinary care. The increasing raising and trading of pigeons unintentionally leads to the risk of spreading infectious diseases, including diseases transmitted from animals to humans (Pedersen et al., 2007). Pigeons are known to be potential hosts for several pathogenic microorganisms, including *Salmonella*, *Escherichia coli*, *Chlamydia* spp., and *Cryptococcus* spp. (Tanaka et al., 2005; Vasconcelos et al., 2018; Karim et al., 2020). Furthermore, due to close contact with humans, pigeons are considered one of the important sources of *Salmonella* infection in humans (Teske et al., 2013; de Oliveira et al., 2018). For example, *Salmonella* strains in pigeon meat can infect humans through the food chain or can be transmitted through direct contact and feces (Teske et al., 2013; de Oliveira et al., 2018).

The use of any antibiotic creates selective pressure favorable to antibiotic-resistant bacteria (Olesen et al., 2020). Therefore, the use and misuse of antibiotics in many countries and regions have made antibiotic resistance a global concern, frequently mentioned in scientific literature (Muteeb et al., 2023; Nammi et al., 2025). Furthermore, the lack of treatment options due to antibiotic resistance is one of the most discussed issues related to human health. Additionally, the misuse and overuse of antibiotics to treat Salmonella infections has led to the emergence of multidrug-resistant (MDR) bacteria (Walther et al., 2017; Xu et al., 2021). To date, studies on the prevalence and antibiotic resistance of bacteria in pigeon farms in Vietnam are limited. Therefore, further studies are needed to clarify the potential risks of bacterial infections, including *Salmonella* spp., to pigeons and human health in Vietnam. This study aimed to determine the prevalence and antibiotic resistance of *Salmonella* spp. in fecal samples isolated from pigeons raised in Northern Vietnam.

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MATERIALS AND METHODS

Ethical approval

The present study was conducted by collecting samples in accordance with the guidelines outlined in the Helsinki declaration and the animal welfare and safety procedures of the Committee on Animal Research and Ethics (CARE), Faculty of Veterinary Medicine, Vietnam National University of Agriculture, Vietnam (Approval No. CARE-2024/01). The authors considered farmers' ethical concerns and consent before conducting the study.

Sampling

In this study, 128 fresh fecal samples were randomly collected from 32 different pigeon farms, as suggested by local veterinarians; each farm is raising at least 1000 pairs of breeding pigeons, in Bac Ninh province, Vietnam, from January to December 2024. The samples were collected according to the guidelines of QCVN 01-83:2011/BNNPTNT of the Ministry of Agriculture and Rural Development (MARD, 2011). Briefly, fresh fecal samples were carefully collected by using sterile spoons. Each sample was placed in a separate sterile sample bag, labeled, stored at 4°C in a dry ice box, and immediately transported to the laboratory of the Department of Veterinary Microbiology and Infectious Diseases, Faculty of Veterinary Medicine, National University of Agriculture, Vietnam, for analysis within 24 hours.

Salmonella isolation

The isolation of *Salmonella* from fecal samples was performed according to the method described in the previous study (Bupasha et al., 2020) with some modifications. Approximately one gram of substance from fecal samples was homologized with buffer peptone water (BPW; Merck, Germany) following the ratio 1:9 and incubated at 37°C within 18-24 hours for pre-enrichment. Next, 0.1 ml of the pre-enriched culture in BPW was added to 10 ml of Rappaport-Vassiliadis soya (RVS) broth (Merck, Germany), followed by further incubation at 42.5°C for 24 hours. A loopful of culture broth was then sampled from the selective enrichment RVS broth, streaked onto xylose lysine deoxycholate agar (XLD; Merck, Germany), and incubated at 37°C for 24 hours. Presumptive black colonies were selected from each plate and cultured on nutrient agar slants (NA; Merck, Germany). A single colony was randomly selected and subjected to biochemical tests such as indole, sugar fermentation, and H2S test, IMViC reaction in Tryptone broth (TB), triple sugar iron agar (TSI), Methyl-red Voges-Proskauer broth (VP-MR broth), and Simmons citrate agar (SCA, Merck, Germany), respectively. All isolates were kept in brain heart infusion broth (BHI; Merck, Germany) supplemented with 50% glycerol at-20°C for subsequent experiments.

Salmonella confirmation

DNA was extracted using the TopPURE® Genomic DNA Extraction Kit (ABT, Vietnam) according to the manufacturer's instructions. The specific primers (Oliveira et al., 2003) corresponding to the following nucleotide sequence based on the *invA* gene, a biomarker for *Salmonella* spp. (Forward: 5'-GTGAAATTATCGCCACGTTCGGGC-3' and Reverse: 5'-TCATCGCACCGTCAAAGGAACC-3'). An expected PCR product of 284 bp was used. The PCR reaction conditions included an initial denaturation at 94ºC for 5 minutes, followed by 35 denaturation cycles at 94ºC for 30 seconds, annealing at 55ºC for 30 seconds, and extension at 72ºC for 45 seconds, followed by a final extension at 72ºC for 10 minutes on a thermocycler (Gene Atlas, Antec Bio, Japan). The reaction components included 12.5 µl of GoTag® Green Master Mix (Promega, USA), 1 µl each of the forward and reverse primers (10 µM), 8.5 µl of purified water, and 2 µl of template DNA. The PCR products were electrophoresed on 1.5% agarose gel supplemented with RedSafe™ Nucleic Acid Staining Solution (Intron, Korea).

Antimicrobial susceptibility test

Antibiotic susceptibility test was examined according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2024). Agar diffusion method was performed on Mueller-Hinton agar (MHA, Merck, Germany) following Bauer et al. (1966) and 14 different antibiotic agents (Oxoid, UK) belong to eight groups were used, including penicillines (ampicillin [10 µg]), carbapenems (meropenem [10 µg]); cephalosporins (cefotaxime [30 µg], ceftazidime [30 µg]); tetracyclines (doxycyline [30 µg]; tetracycline [30 µg]); aminoglycosides (gentamicin [10 µg], streptomycin [10 µg]); phenicols (chloramphenicol [30 µg]); quinolones (nalidixic acid [30 µg], ciprofloxacin [5 µg], norfloxacin [10 µg]); sulfonamides (sulfonamides [300 µg], trimethoprim/sulfamethoxazole [1.25/23.75 µg]). The *Escherichia coli* ATCC 25922 strain is used for quality control. An isolate was determined to be resistant or multidrug-resistant to antibiotics based on the definition of Magiorakos et al. (2012).

Data analysis

The data was entered and calculated using Microsoft Excel 2016 software.

RESULTS

After conducting biochemical and molecular tests, 31.3% (40/128) of fecal samples were *Salmonella*-positive (Figure 1). Antibiotic resistance rates of the isolated *Salmonella* strains were presented in Table 1. The isolates exhibited the highest resistance rate to tetracycline (67.5%), followed by ampicillin and sulfonamides (62.5%). Cefotaxime, streptomycin, and trimethoprim/sulfamethoxazole were resistant at rates of 52.0%, 47.5%, and 45.0%, respectively. The *Salmonella* strains showed resistance to ciprofloxacin (20.0%), gentamicin (25.0%) and doxycycline (25.0%), nalidixic acid (32.5%), and

chloramphenicol (35.0%). No *Salmonella* strains were found to be resistant to ceftazidime, meropenem, and norfloxacin. The antibiotic resistance patterns of the *Salmonella* isolates are presented in Table 2. The *Salmonella* strains exhibited 26 different antibiotic resistance patterns. Of which, resistance to 1-2 and 3-5 antibiotics was found in eight and twelve of the isolates, respectively. There were eleven and six of the isolates that exhibited resistance to 6-8 and 9-11 kinds of antibiotics, respectively. The results in Table 3 indicated that 92.5% (37/40) *Salmonella* isolates were resistant to at least one antibiotic, of which 70.0% (28/40) isolates were identified as MDR. Among the MDR isolates, resistance to 3-4 antibiotic classes, 5-6 antibiotic classes, and ≥7 antibiotic classes was found in eleven (27.5%), twelve (30.0%), and five (12.5%) out of the *Salmonella* isolates, respectively.

Table 1. Antibiotic resistance status of the isolated *Salmonella* spp. from fecal samples of pigeons farmed in Bac Ninh, Vietnam, in 2024

Antibiotic groups	Antibiotic	Resistance n (%)	Intermediate n (%)	Susceptible n (%)
Beta-lactams	Ampicillin	25 (62.5)	2 (5.0)	13 (32.5)
	Ceftazidime	0 (0.0)	0 (0.0)	40 (100)
	Cefotaxime	21 (52.5)	1 (2.5)	18 (45.0)
	Meropenem	0 (0.0)	3 (7.5)	37 (92.5)
aminoglycosides	Gentamicin	10 (25.0)	2 (5.0)	28 (70.0)
	Streptomycin	19 (47.5)	3 (7.5)	18 (45.0)
Quinolones	Nalidixic acid	13 (32.5)	4 (10.0)	23 (57.5)
	Ciprofloxacin	8 (20.0)	3 (7.5)	29 (72.5)
	Norfloxacin	0 (0.0)	2 (5.0)	38 (95.0)
Tetracyclines	Doxycycline	10 (25.0)	2 (5.0)	28 (70.0)
	Tetracycline	27 (67.5)	3 (7.5)	10 (25.0)
Phenicols	Chloramphenicol	14 (35.0)	3 (7.5)	23 (57.5)
Sulfonamides	Sulfonamides	25 (62.5)	1 (2.5)	16 (40.0)
	Trimethoprim/Sulfamethoxazole	18 (45.0)	2 (5.0)	20 (50.0)

Table 2. Antibiotic resistance patterns of the isolated *Salmonella* spp. from fecal samples of pigeon farmed in Bac Ninh, Vietnam, in 2024

Order	Antibiotic resistance patterns	Number of antibiotic resistances	Number of strains
1	Susceptible	0	3
2	AMP	1	1
3	TET	1	4
4	SXT, TET	2	3
5	SUL, SXT, TET	3	1
6	SUL, SXT, STM, TET	4	1
7	AMP, CTX, CHL, STM	4	1
8	AMP, CTX, SUL, DOX	4	2
9	AMP, SUL, STM, GEN	4	1
10	AMP, CTX, CHL, STM, GEN	5	1
11	AMP, CTX, SUL, TET, DOX	5	1
12	AMP, CTX, CHL, SUL, SXT	5	2
13	AMP, CTX, SXT, STM, NAL	5	1
14	AMP, SUL, STM, TET, NAL	5	1
15	AMP, CHL, SUL, SXT, TET, DOX	6	2
16	AMP, CTX, SUL, SXT, STM, TET	6	2
17	AMP, CTX, CHL, SUL, GEN, TET, DOX	7	1
18	AMP, CHL, SUL, SXT, STM, GEN, TET, DOX	8	1
19	AMP, CTX, CHL, SUL, STM, GEN, TET, NAL	8	2
20	AMP, CTX, SUL, STM, GEN, TET, NAL, CIP	8	1
21	AMP, CTX, SUL, STM, TET, DOX, NAL, CIP	8	1
22	AMP, CTX, SUL, SXT, STM, TET, DOX, NAL	8	1
23	AMP, CTX, CHL, SUL, STM, TET, DOX, NAL, CIP	9	2
24	AMP, CTX, SUL, STM, GEN, TET, DOX, NAL, CIP	9	1
25	AMP, CTX, SUL, SXT, STM, TET, DOX, NAL, CIP	9	1
26	AMP, CHL, SUL, SXT, STM, GEN, TET, DOX, NAL, CIP	10	1
27	AMP, CTX, CHL, SUL, SXT, STM, GEN, TET, DOX, NAL, CIP	11	1

AMP: Ampicillin; CTX: Cefotaxime; CHL: Chloramphenicol; CIP: Ciprofloxacin; DOX: Doxycycline; GEN: Gentamicin; NAL: Nalidixic acid; NOR: Norfloxacin; STM: Streptomycin; STX: Trimethoprim/Sulfamethoxazole; SUL: Sulfonamides; TET: Tetracycline

Table 3. Number of the multi-drug resistant *Salmonella* strains isolated from fecal samples of pigeons farmed in Bac Ninh, Vietnam in 2024

Order	Number of antibiotic resistance groups	Number of exhibited strains	
		Number	Percentage
1	Susceptible	3	7.5
2	Resistance to 1 - 2 classes	9	22.5
3	Resistance to 3 - 4 classes	11	27.5
4	Resistance to 5 - 6 classes	12	30.0
5	Resistance to ≥ 7 classes	5	12.5

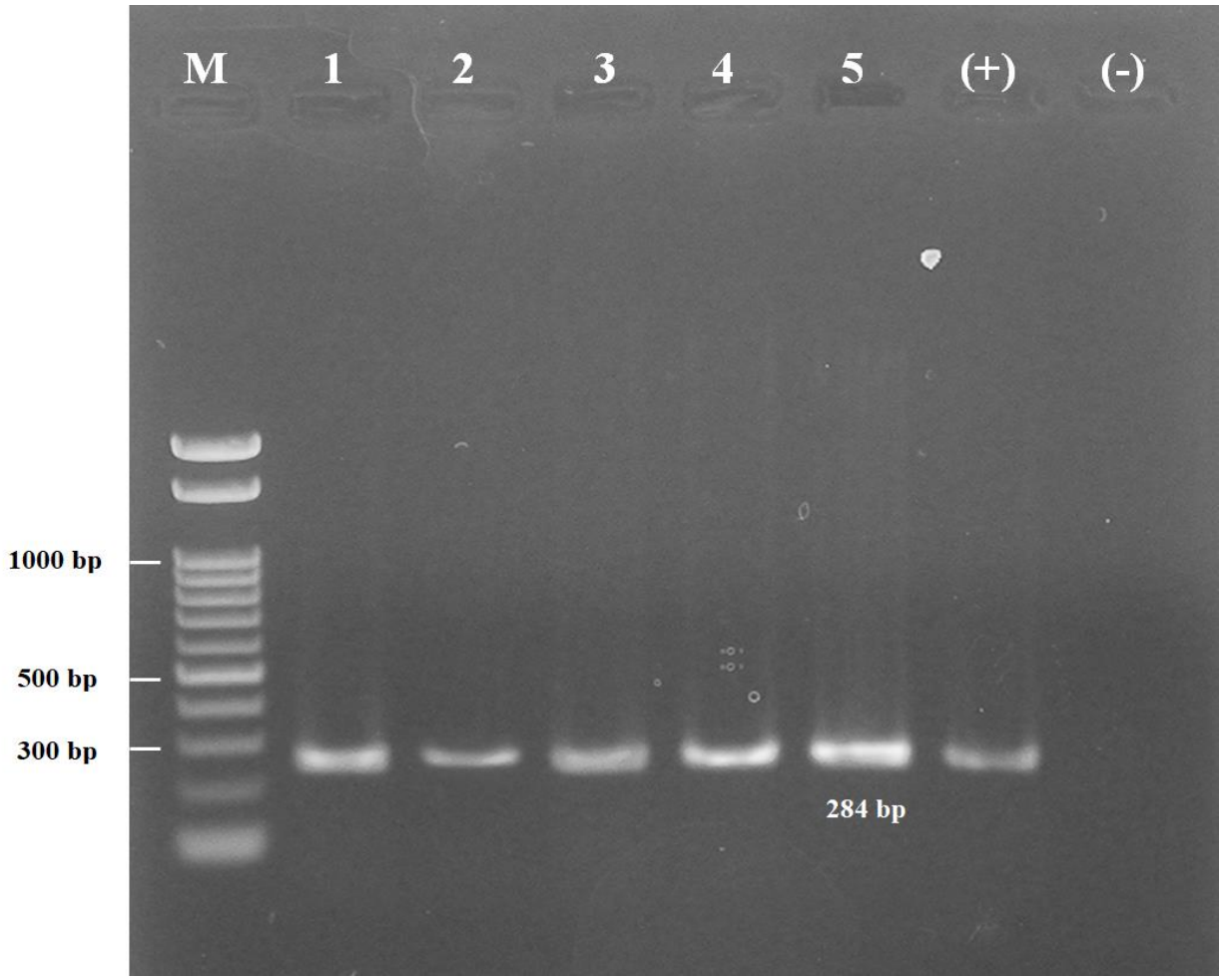

Figure 1. Agarose gel electrophoresis of *invA* gene in *Salmonella* strains isolated from fecal samples of pigeons farmed in Bac Ninh, Vietnam, in 2024. M: DNA maker (100 bp), Lane 1-5: *invA* positive (284 bp), (+): positive control, (-): negative control.

DISCUSSION

In this study, the *invA* gene was amplified in all isolates *Salmonella*, in agreement with the studies conducted in Egypt (El-Sebay et al., 2017; Hagag et al., 2022), Indonesia (Wibisono et al., 2021), and Iran (Mashayekh et al., 2022). The *invA* gene is widely used to identify *Salmonella* species in various samples and is associated with intestinal invasion in animals (Oliveira et al., 2003; El-Sebay et al., 2017). The *invA* gene is also a suitable marker for molecular analyses and preliminary phylogenetic analysis of *Salmonella* in epidemiological studies (McWhorter et al., 2019; Fadipe and Hölzle, 2025). The rate of *Salmonella*-positive (31.1%) is consistent with rates ranging from 22.22% to 37.5% in cloacal swab and fecal samples of pigeons in previous studies in Bangladesh (Hosain et al., 2012; Karim et al., 2020; Bupasha et al., 2021). However, the isolation rates of *Salmonella* species were only about 0.81% and 2.0% in pigeons in other studies in Brazil and Egypt (Ruben et al., 2018; Hagag et al., 2022), respectively. In addition, *Salmonella* was detected in pigeon organ samples ranging from 3.5% in Egypt (Yousef and Mamdouh et al., 2016), 5.47% in Poland (Kaczorek-Łukowska

et al., 2021), 8.0% in India (Dutta et al., 2013), 14.9% in China (Zhang et al., 2024), to 28.57% in Bangladesh (Raman et al., 2016). The prevalence of *Salmonella* in pigeons varied across studies in different countries, which may be related to different sampling methods. However, the high positive rate of *Salmonella* in pigeon fecal samples in this study may be due to poor sanitary conditions and inadequate hygiene practices, as well as cross-contamination between high-density cages at the farms. Therefore, this underscores the need for stricter hygiene measures at pigeon farms in the study area.

Antibiotics are the most common therapy for treating bacterial diseases, including *Salmonella* infections in pigeons (Tang et al., 2023; Zhang et al., 2024). In veterinary medicine, the widespread and continuous use of antibiotics has exacerbated antibiotic resistance in bacterial strains derived from animals and livestock (Tang et al., 2023). High resistance rates of *Salmonella* to tetracycline, ampicillin, sulfonamide, and streptomycin have been noted in previous studies in India (Dutta et al., 2013), Bangladesh (Karim et al., 2020; Bupasha et al., 2021), and China (Zhang et al., 2024). The high levels of resistance to these antibiotics may be due to their widespread use in the treatment and prevention of diseases in both veterinary and human medicine worldwide (Bupasha et al., 2021). In the current study, 32.5% of the *Salmonella* isolates were resistant to nalidixic acid. The results of the current study are lower than previous reports conducted in India (Dutta et al., 2013) and Bangladesh (Saifullah et al., 2016; Bupasha et al., 2021), which reported that 50.0-72.4% of the isolates were resistant to this antibiotic, respectively. The rate of *Salmonella* resistance to ciprofloxacin was similar to that in previous studies, as about 10.0% in China (Zhang et al., 2024), and 28.6% in Egypt (Yousef and Mamdouh et al., 2016). However, another study in Bangladesh (Saifullah et al., 2016; Karim et al., 2020) and India (Dutta et al., 2013) reported that all *Salmonella* strains isolated from pigeons were susceptible to this antibiotic. All *Salmonella* strains in the present study were susceptible to norfloxacin. However, approximately 8.33%, 15.6%, and 20.0-29.0% of *Salmonella* strains derived from pigeons in previous studies in India (Dutta et al., 2013), Poland (Kaczorek-Łukowska et al., 2021), and Egypt (Yousef and Mamdouh et al., 2016), respectively, exhibited resistance to norfloxacin.

The gentamicin resistance rate in this study was consistent with the rate of 22.0% in Bangladesh (Saifullah et al., 2016). However, resistance to gentamicin of *Salmonella* varied significantly between countries, ranging from 8.33% in India (Dutta et al., 2012) to 100% in China (Zhang et al., 2024). Similarly, the trimethoprim/sulfamethoxazole resistance of *Salmonella* strains was reported to be under 10.0% in China (Zhang et al., 2024), ranging from 21.8% to 25.0% in Poland and India (Dutta et al., 2012; Kaczorek-Łukowska et al., 2020), approximately 50.0% to 55.6% in Egypt (Yousef and Mamdouh et al., 2016; Hagag et al., 2022), and up to 86.2% in Bangladesh (Saifullah et al., 2016). The rate of doxycycline resistance was consistent with the rate of 22.2% in a previous study conducted in Egypt (Hagag et al., 2022), but lower than the rate of 37.5% in a study conducted in Poland (Kaczorek-Łukowska et al., 2020). In the current study, chloramphenicol, a drug banned for use in livestock and veterinary medicine in Vietnam, was resistant by 35.0% of the isolates. This may be due to the widespread use of other phenicol antibiotics, such as florfenicol and thiamphenicol, in livestock and veterinary medicine in Vietnam. The rate of cefotaxime resistance was 52.5%, which could comparable to results from previous studies conducted in India (Dutta et al., 2012), in Egypt (Yousef and Mamdouh et al., 2016; Hagag et al., 2022), and Bangladesh (Bupasha et al., 2020). Conversely, all isolates were susceptible to ceftazidime and meropenem, consistent with those reported by Zhang et al. (2024) in China. This could be due to the limited use of these two drugs in animal husbandry and veterinary medicine because their high cost makes them inaccessible in Vietnam.

The frequent use of antibiotics in livestock and veterinary medicine for disease prevention and treatment is a significant factor leading to the emergence of antibiotic-resistant bacteria, which are then transmitted to humans through the food chain (Hosain et al., 2012; Tang et al., 2023). In this study, approximately 70% of the isolated *Salmonella* strains were identified as multidrug-resistant (MDR). However, this is not surprising given the high rates of MDR in pigeon-derived *Salmonella* strains in previous studies. The proportion of isolates exhibiting MDR ranged from 54.54% to 96.6% in studies conducted in Bangladesh (Bupasha et al., 2020; Karim et al., 2020) and 90.62% in China (Zhang et al., 2024). Currently, due to limited vaccine and antibiotic supplies in the pigeon breeding industry, infections caused by MDR bacteria can lead to ineffective treatment in this species (Kaczorek-Łukowska et al., 2021; Zhang et al., 2024). The situation underscores the need for continuous monitoring of antibiotic resistance in pigeon populations and the cautious use of antibiotics.

CONCLUSION

The results of the current study indicated a high prevalence of antibiotic-resistant *Salmonella* in pigeon farms of Vietnam. Furthermore, many isolated bacterial strains exhibited resistance to multiple antibiotics. This underscores the need for monitoring measures to limit the indiscriminate use of antibiotics in pigeon farming and thus prevent the spread of antibiotic-resistant bacteria. In addition, more extensive studies on other pigeon diseases are needed to support the management and development of the pigeon farming industry in Vietnam.

DECLARATIONS

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Authors' contributions

Chu Thi Thanh Huong designed the investigation and methodology and contributed to the manuscript. Vu Thi Thu Tra and Truong Lan Oanh participated in doing the experiments and analyzing the data. Truong Ha Thai was responsible for supervision, methodological support, and primarily for writing the manuscript. All authors revised and approved the final edition of the manuscript before publication in the present journal.

Availability of data and materials

All data are included in the submitted paper and will be available upon reasonable requests from the corresponding author.

Ethical considerations

This article was written originally without any copy from published articles and books. No AI tools were used for conducting and preparing the current study.

Competing interests

The authors declared no conflict of interest.

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